Variation among and within Smooth Bromegrass Collections from Rural Cemeteries

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ABSTRACT

Smooth bromegrass (*Bromus inermis* Leyss.) is poorly adapted to management-intensive rotational grazing because of slow and limited regrowth potential. In an effort to find existing germplasm with tolerance to frequent cutting, smooth bromegrass germplasm was collected from fence and sod habitats of 30 rural cemeteries in Iowa, Minnesota, and Wisconsin. Ramets of 25 clones from each habitat of each cemetery were transplanted into a replicated and randomized experiment at Arlington, WI, and evaluated from 1999 to 2002. Within-population genotypic variance was greater in sod populations for plant height and diameter. Across cemeteries, genotypic variances for regrowth vigor of sod and fence populations were positively correlated. These two results suggest that a large amount of genotypic variability is being maintained at some cemeteries by migration into sod populations and disruptive selection favoring different genotypes in the two habitats. Fence populations averaged 7.6% higher in reproductive forage yield, 9.5% higher in vegetative forage yield, 6.0% taller, 8.4% wider plant diameter, 4.7% higher regrowth vigor, and 6.9% higher frequent-harvest forage yield than sod populations. Sod populations tended to be more variable among cemeteries than fence populations, suggesting greater adaptive responses to selection pressure. Two sod populations were highly unusual, one with unusually fast regrowth arising from tillers that initiated obvious growth within 24 h after apical dominance was removed, the other with extremely high reproductive forage yield, but low regrowth vigor. This germplasm may have value in the development of smooth bromegrass germplasm with improved tolerance to frequent cutting or grazing.

SMOOTH BROMEGRASS is an important forage grass in much of temperate North America. It is preferentially adapted to hay management and favored by infrequent cutting, relatively high cutting heights, and high nitrogen fertility (Casler and Carlson, 1995). Conversely, smooth bromegrass is not well adapted to frequent defoliation, whether by cutting (Smith et al., 1973) or by grazing (Casler et al., 1998), or to low defoliation heights (Lawrence and Ashford, 1969; Raese and Decker, 1966; Smith et al., 1973). Unlike many other cool-season forage grasses, forage production of smooth bromegrass is not stimulated by defoliation, regardless of the growth stage (Harrison and Romo, 1994; Lawrence and Ashford, 1969). Smooth bromegrass stands decline under rotational grazing, an effect that is magnified by increasingly intensive grazing (Bittman and McCartney, 1994). Smooth bromegrass is used primarily for infrequent hay harvests, soil conservation, or other situations that are characterized by relatively low levels of management.

Regrowth and persistence of smooth bromegrass is limited largely by the timing of new tiller development.

Development of new tillers in smooth bromegrass is largely determinant, with synchronized elevation and elongation of new apical meristems above the soil surface (Krause and Moser, 1977). Cutting or grazing before new tillers have developed sufficiently reduces regrowth and persistence (Eastin et al., 1964; Reynolds and Smith, 1962). During reproductive development, this critical time occurs from culm elongation to late heading. Cutting before culm elongation (to avoid removal of apical meristems) or well after heading (when new tillers have begun to emerge) leads to increased forage yields and persistence (McElgunn et al., 1972; Paulsen and Smith, 1968). Apical dominance in smooth bromegrass is strong until anthesis, when auxin activity declines and tillering is normally resumed (Eastin et al., 1964). Because smooth bromegrass produces true culms with elevated apical meristems upon regrowth, timing of subsequent harvests may also be critical for smooth bromegrass regrowth and persistence. Regrowth of smooth bromegrass is not closely related to carbohydrate reserves in roots and crowns (Eastin et al., 1964; Paulsen and Smith, 1969; Raese and Decker, 1966; Reynolds and Smith, 1962).

Use of smooth bromegrass in grass–legume mixtures is limited by its synchronized tiller development. First harvest in smooth bromegrass–alfalfa (*Medicago sativa* L.) mixtures typically occurs during the critical late-jointing phase. This suppression of smooth bromegrass regrowth potential, combined with shading from the rapidly recovering alfalfa canopy leads to rapid smooth bromegrass stand losses (Casler, 1988; Smith et al., 1973). Breeding and selection for persistence of smooth bromegrass in mixture with alfalfa under a three- or four-cut management system has been somewhat successful. Populations selected for persistence had 40% greater ground cover and 42% faster recovery after cutting than unselected cultivars (Casler, 1988). The cultivar Alpha, a product of this program, had 10% greater survival after 2 yr in mixture with alfalfa across five locations than the second-ranked cultivar (Casler, 1988; Casler and Walgenbach, 1990). Despite these successes, smooth bromegrass cultivars, including Alpha, have relatively low persistence under management-intensive rotational grazing systems (Casler et al., 1998).

In June 1995, while visiting my maternal grandparents' gravesite, I discovered a thick and vigorous stand of smooth bromegrass growing in a sod dominated by Kentucky bluegrass (*Poa pratensis* L.). The turf was well-managed and frequently mowed to maintain its visual appearance, suggesting that this smooth bromegrass population may have adaptive traits allowing it to survive and vegetatively reproduce under frequent defoliation. Subsequent investigations identified numer-

Abbreviations: NPD, normalized phenotypic distance; PD, Euclidean phenotypic distance.

Table 1. Location information for 30 rural cemeteries which provided smooth bromegrass germplasm.

Site No.	Site ID	Cemetery name	Nearest town	N latitude	W longitude
1	W12Z	Sauk Prairie	Prairie du Sac, WI	43°19′	89°46′
2	W15173	Bethel	Columbus, WI	43°21′	89°01′
3	W151VV	Unknown	East Bristol, WI	43°14′	89°08′
4	W81PR	Pleasant Ridge	Bloomington, WI	42°52′	90°51′
5	W12SLR	Town of Delton	Dellwood, WI	43°33′	89°47′
6	W69D	Belleville (S)	Belleville, WI	42°52′	89°32′
7	W69A	Belleville (N)	Belleville, WI	42°52′	89°32′
8	WDEXR	Exeter	Attica, WI	42°47′	89°31′
9	WPB69	Paoli	Paoli, WI	42°56′	89°32′
10	WA162	Sand Creek	Four Corners, WI	44°03′	90°56′
11	W108A	Burr Oak	Burr Oak, WI	44°04′	91°04′
12	WE14	Basswood Union Church	Basswood, WI	43°16′	90°30′
13	MW5317	St. John's Trinity L. C.	Janesville, MN	44°10′	93°32′
14	MRC19	Unknown	Fairfax, MN	44°31′	94°41′
15	MRO212	Olivia	Olivia, MN	44°47′	95°00′
16	MR267	Morgan City	Morgan, MN	44°24′	94°56′
17	MC1320	Havelock Township	Aggie, MN	45°04′	95°34′
18	MC3520	Immanuel L. C.	Watson, MN	45°03′	95°45′
19	ML3120	Unknown	Lac qui Parle, MN	44°59′	95°44′
20	MYM5C3	Camp Release	Clarkfield, MN	44°51′	95°43′
21	MYMSA15	Hillside	Echo, MN	44°41′	95°24′
22	I151BP	Bowen's Prairie	Cascade, IA	42°15′	91°04′
23	IB7123.5	Salem	Van Horne, IA	42°01′	92°02′
24	IM330S	Marietta	Marietta, IA	42°04′	92°58′
25	IWWT	Primitive Baptist Church	Winterset, IA	41°14′	94°01′
26	IMD20	McDonald Chase	Winterset, IA	41°15′	94°06′
27	IB210MS	Hillsdale	Madrid, IA	41°51′	93°44′
28	IB190169	Beaver Creek	Beaver, IA	42°06′	94°08′
29	ISHI35	Sheffield	Roland, IA	42°07′	93°33′
30	ISU210C	Center Grove	Cambridge, IA	41°52′	93°28′

ous such cemeteries in Minnesota, Wisconsin, and Iowa. Many of these cemeteries also had a wire fence or border area dominated by smooth bromegrass. Fence and border areas were unmanaged, suggesting that fence and border populations of smooth bromegrass may have been subjected to different natural selection pressures than sod populations.

The objective of this study was to characterize smooth bromegrass plants collected from sod and fence habitats of 30 well-managed rural cemeteries in Iowa, Minnesota, and Wisconsin. It is impossible to know the origin of founder plants of these fence and sod populations or to be certain that paired populations from a cemetery are of similar origin. Smooth bromegrass was used extensively in rural areas of these three states in the 1930s (Casler and Carlson, 1995) and may have been the major component of rural cemetery sods in this region. The advent of turfgrass breeding in the 1950s and the development of seed markets and seeding methods led to widespread mechanical renovation of turf areas. Many smooth bromegrass populations likely survived this renovation, resulting in remnant survivors in these rural cemeteries. Fence populations would have had a distinct advantage over sod populations because of less intensive interspecific competition and lack of mowing management. Thus, it is possible that fence and sod populations have evolved into morphologically and/or adaptively different phenotypes.

MATERIALS AND METHODS

Smooth bromegrass plants were collected from 30 cemeteries in Minnesota, Wisconsin, and Iowa in 1995 and 1996 (Table 1, Fig. 1). Rural cemeteries were located on plat maps of Chippewa, Lac Qui Parle, Redwood, Renville, and Yellow Medicine Counties in Minnesota; Adams, Dane, Grant, Iowa,

Portage, Richland, and Wood Counties in Wisconsin; and Benton, Boone, Madison, Marshall, and Story Counties in Iowa. Each cemetery has been in existence since the mid- to late 19th century. All rural cemeteries in these 17 counties were visited in June to August 1995 or June 1996. Smooth bromegrass plants were collected only from cemeteries with the
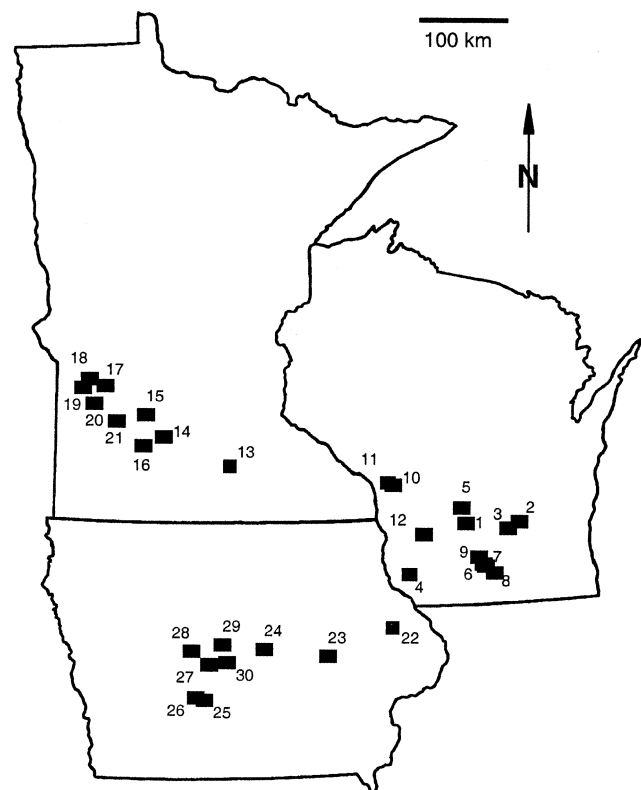

Fig. 1. Geographic distribution of 30 rural cemeteries, the source of smooth bromegrass germplasm from fence and sod habitats.

following characteristics: (i) a well-managed turf, dominated by Kentucky bluegrass, with few obvious weeds and showing no evidence of infrequent or lax mowing management, (ii) a reasonably vigorous stand of smooth bromegrass in the sod, (iii) a good stand of uncut smooth bromegrass in the fence or border area. Approximately one-third of all rural cemeteries in these 17 counties met each of these criteria.

Forty smooth bromegrass plants each from the sod and the fence were collected. Each plant was represented by a single live tiller. Smooth bromegrass plants were collected largely at random from the entire colonized region of the sod or from the entire length of the fence or border area. For sod collections, each tiller was collected a minimum distance of 2 m apart. For fence collections, the entire fence or border area was divided into 40 approximately equal segments and one tiller was collected from each segment. All tillers were potted in the greenhouse and subsequently transplanted to a field at Arlington, WI, in May of the year following their collection. Plants were unreplicated and spaced on 0.9-m centers. The clonal nursery was fertilized twice per year with 56 kg N ha^{-1}, mowed three times per year, and kept weed-free by preemergence herbicides (Falkner and Casler, 1998) and hand weeding.

Despite all efforts to create a favorable environment for collected tillers, some plants did not survive transplanting in the greenhouse or the field. Twenty-five random plants of each population (30 sod populations and 30 fence populations) were randomly selected from among the survivors by taking the first 25 plants in each row of the clonal nursery. Two clonal ramets of each clone were transplanted to a split-split-plot randomized complete block experiment with two replicates in May 1998. Whole plots were represented by the 30 cemeteries, subplots were a single row of 25 plants from one of the two habitats (fence or sod), and sub-subplots were individual clones within each of the 60 populations. All plants were spaced on 0.9-m centers. The soil was a Plano silt loam (fine-silty, mixed, superactive, mesic, Typic Argiudolls). Clonal propagules were cylinders 10 cm in diameter, approximately 10 cm deep, and containing 10 to 15 live tillers. All transplants were watered several times immediately after transplanting and those with poor establishment were replaced with another ramet of the same clone within the first 4 wk after transplanting.

The experiment was fertilized with 112 kg N ha^{-1} in early spring and following the first harvest of 1999 and 2000. Plant height was measured on all plants in early June, just after all plants were fully headed. Forage from each row of 25 plants was harvested in bulk by a flail harvester and converted to a dry matter basis by a bulk dry matter sample taken from the entire field. Cutting height was 7 cm. Plant diameter was measured at the widest part of the crown immediately after first harvest. Regrowth vigor was measured as canopy height approximately 2 wk after first harvest. Forage was harvested again in September as described for first harvest.

The experiment was fertilized with 45 kg N ha^{-1} in early spring 2001 and 2002. Each plot was harvested with a flair harvester when the grass canopy was approximately 20 cm tall. Five harvests were made in each year, all when the canopy height was 15 to 20 cm. Cutting height and dry matter determination were as described above. The experiment was fertilized with 45 kg N ha^{-1} after each of the first four harvests. Total frequent-harvest forage yield was the sum of dry matter yield across five harvests (early May to late October).

Plant height, plant diameter, and regrowth vigor were subjected to ANOVA within each of the 60 populations. Clones, blocks, and years were all assumed to have random effects. Variance components for clones were estimated by equating mean squares to their expectations (Gaylor et al., 1970).

Pooled clone variance components for states and habitats were created from pooled analyses of variance in which states and habitats were considered to have fixed effects. Confidence intervals for clone variance components were computed according to Milliken and Johnson (1984).

Subplot means for plant height, plant diameter, and regrowth vigor and raw data for reproductive forage yield, vegetative forage yield, and frequent-harvest forage yield were subjected to ANOVA in which states and habitats were fixed, while sites within states, blocks, and years were random. Variance components for sites within states were computed as described above. Fence vs. sod comparisons were made by contrasts. A combined ANOVA was computed for total forage yield across 4 yr, based on the total of all harvests within each year, considering total forage yield for frequent and infrequent harvest managements to be a single variable. The population × year interaction (3 df) was partitioned into population × management (1 df) and population × year/management (2 df).

The 60 population means for all six variables were subjected to principal components analysis. Euclidean phenotypic distance (PD) values were computed among all 60 populations by the formula

$$\mathrm{PD} = \sum_{k=1}^{6} (M_{ik} - M_{jk})^2,$$

where M_{ik} and M_{jk} are means for populations i and j, respectively, and variable k; summation was across six variables ($k = 1,...,6$). Population means were standardized to $\mu = 0$ and $\sigma = 1$ before computation of PD. Phenotypic distances were converted to normalized phenotypic distances (NPD) by dividing each value by the mean phenotypic distance. The NPD was adapted from Smouse and Peakall (1999).

RESULTS AND DISCUSSION
Within-Population Genotypic Variability

Clone × year interaction was significant ($P < 0.05$) in 16, 22, and 3 populations for plant height, plant diameter, and regrowth vigor, respectively (data not shown). Of these significant clone × year interactions, only eight of the clone × year variance components exceeded their respective clone variance component—five for plant diameter and three for regrowth vigor. There was no relationship between state or site and the significance or magnitude of clone × year variance components. Therefore, clone × year interactions were largely unimportant relative to clone main effects.

Within-population genotypic variance was significant ($P < 0.10$) for at least one trait (plant height, plant diameter, and regrowth vigor) in 59 of 60 populations (data not shown). However, for three of these 59 populations, all traits failed to show significant within-population variance at the $P < 0.05$ level. Thus, four populations (WI-3-sod, WI-9-fence, WI-10-fence, and IA-28-fence) appear to have very low amounts of genotypic variation. This suggests that these populations were founded by a relatively small number of genotypes or there has been considerable natural selection and mortality leading to a very small number of genotypes remaining in these populations. Many of the 25 clones of these four populations may be vegetative propagules of each other. Because smooth bromegrass can spread by rhizomes, individual genotypes have the potential to spread across a

Table 2. Pooled variance components for clones (with 95% confidence intervals in parentheses) within smooth bromegrass populations from each state and habitat (fence vs. sod).

State†	Plant height		Plant diameter		Regrowth vigor	
	Fence	Sod	Fence	Sod	Fence	Sod
				cm		
Wisconsin	672	1165	673	1040	47	34
(288)	(444, 937)	(840, 1554)	(435, 948)	(687, 1451)	(17, 81)	(2, 68)
Minnesota	1103	2192	948	1363	48	60
(216)	(800, 1465)	(1675, 2828)	(643, 1304)	(974, 1826)	(13, 86)	(15, 108)
Iowa	909	1010	1256	1128	62	40
(216)	(647, 1207)	(707, 1354)	(917, 1646)	(775, 1527)	(19, 107)	(5, 78)
Pooled	2683	4367	2877	3532	157	134
(720)	(2216, 3194)	(3694, 5111)	(2353, 3446)	(2887, 4233)	(94, 223)	(69, 201)

† Degrees of freedom in parentheses. All mean squares associated with variance components were significant at $P < 0.01$.

large area, resulting in multiple samples of a single clone even with a careful and systematic sampling strategy.

There were 51, 51, and 27 populations that showed significant within-population genotypic variation for plant height, plant diameter, and regrowth vigor, respectively (data not shown). There were few differences in within-population variation between fence and sod populations, partly because of low degrees of freedom. Furthermore, for plant height and diameter, there was no relationship between the variance component for fence and sod populations; highly variable sod populations were not associated with highly variable fence populations for these two traits. However, the opposite was observed for regrowth vigor; variance components for sod and fence populations were positively correlated ($r = 0.37$, $P < 0.05$). This is circumstantial evidence for disruptive selection and migration between fence and sod habitats in cemeteries with large amounts of variability. Disruptive selection, favoring different genotypes in fence and sod habitats, and migration of alleles between habitats would combine to maintain large amounts of genotypic variance within populations sampled from each habitat.

For regrowth vigor, pooled variance components were nearly identical for fence and sod populations (Table 2). However, for plant height and diameter, sod populations had generally greater genotypic variability than fence populations, suggesting the possibility that migration may be maintaining large amounts of genotypic variability in some sod populations. Migration from fence to sod populations can occur by rhizome growth or seed dispersal followed by seedling recruitment, whereas rhizome growth is the only mechanism for migration from sod to fence populations. Migration from sod to fence by rhizomes is unlikely, because all fence-lines contained solid and vigorous populations of smooth bromegrass tillers. Thus, migration from fence to sod may be the mechanism maintaining larger amounts of genotypic variability for some traits in sod populations compared with fence populations.

Seed dispersal and seedling recruitment may also be a source of migratory alleles from smooth bromegrass populations outside the cemetery. Numerous rodents and birds are known to consume seeds of *Bromus* species (Martin et al., 1951) and grass seeds may survive fermentation in digestive tracts of birds and small mammals (Pakeman et al., 1999; Stiles, 1992). Thus, the po-

tential exists for seed deposition into a moist, nutrient-rich microenvironment. Frequent mowing of the sod habitat may favor seedling recruitment from either the fence habitat or external seed sources. Light is critical for both germination and development of perennial grass seedlings (Grime, 1966), favoring seedling recruitment in the sod habitat over the fence habitat. Seedling recruitment is highly favored by disturbances that create patches of open ground, however small (Burke and Grime, 1996; Thompson et al., 1996). Numerous opportunities exist for such disturbances within these sod habitats, increasing the likelihood that alleles are migrating from either the fence habitat or external sources into the sod populations.

External pollen may contribute to migration of alleles into the fence population, but this is likely to be a small source of genotypic variability because smooth bromegrass pollen rarely travels over large distances (Hittle, 1954; Knowles, 1969) and there were no additional sources of smooth bromegrass pollen within visual sight of these cemeteries. Furthermore, migration of alleles via pollen would require the additional step of seedling recruitment, which is unlikely in the fence habitat because of competition from existing vegetation and low light conditions at the soil surface.

Among-Population Genotypic Variability

Population × year interaction was significant for reproductive forage yield, plant height, and plant diameter ($P < 0.05$). However, for these three traits, the variance component for populations was 6.8 to 9.9 times higher than the population × year interaction variance component. Therefore, population × year interactions were relatively unimportant. All variance analyses were based on the expected mean squares including population × year interaction as a source of variation. All analyses of means and effects were based on means across years.

For total yield analyzed across all 4 yr, 89% of the overall population × year interaction was due to differences in management between 1999–2000 and 2001–2002. The phenotypic correlations between years ($n = 60$) were $r = 0.89$ ($P < 0.01$) for 1999 vs. 2000 (two harvests), $r = 0.63$ ($P < 0.01$) for 2001 vs. 2002 (five harvests), and $r = 0.21$ to 0.28 ($0.03 < P < 0.10$) for 1999 or 2000 vs. 2001 or 2002. Because there were no differences in weather patterns or fertilization levels
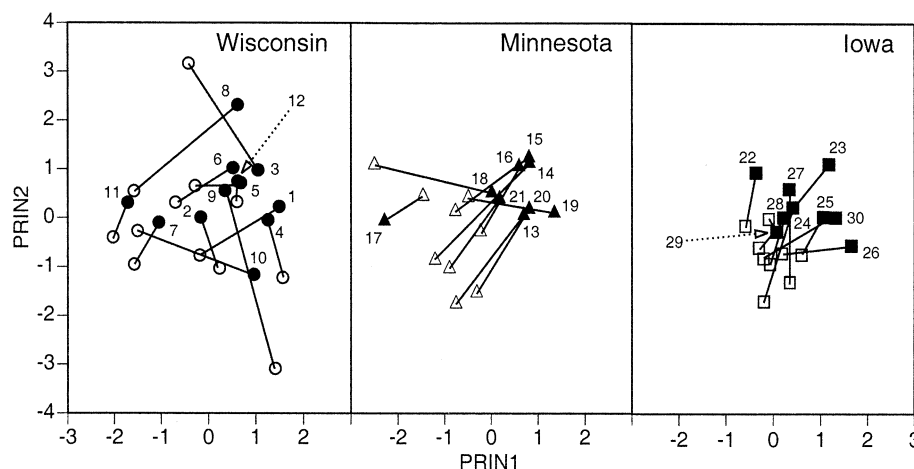
Fig. 2. Plots of the first two principal components (PRIN 1 and PRIN2) for 60 smooth bromegrass populations. Fence populations are identified by closed symbols and sod populations by open symbols. Each fence population is labeled with the site number from Fig. 1 and Table 1. Euclidean vectors connect paired fence and sod populations from a single site.

between 1999–2000 and 2001–2002, this correlation structure is likely due to the change in harvest management. These results suggest that drastic changes in harvest frequency can significantly alter the ranking and relative differences among smooth bromegrass populations.

Populations differed for all six traits at $P < 0.01$. Principal components analysis resulted in the first two components that described 68% of the variability among populations (data not shown). Component 1 (50%) was largely associated with high forage yield for both harvests, tall plants, and highly spreading plants. These four variables were all positively correlated with each other ($r = 0.47$ to 0.74, $P < 0.01$). Component 2 (18%) was largely associated with high regrowth vigor and high frequent-harvest forage yield. Regrowth vigor was not correlated with vegetative forage yield, plant height, and plant diameter, and was negatively correlated with reproductive forage yield ($r = -0.28$, $P < 0.05$). Frequent-harvest forage yield was correlated only with reproductive forage yield under infrequent harvest ($r = 0.29$, $P < 0.05$). This covariance structure suggests that populations with high reproductive forage yield have partitioned insufficient carbohydrate reserves into crowns and roots where they would be needed for rapid regrowth. Following Cut 1, rapid regrowth (measured by regrowth vigor) is not correlated with extent of regrowth (measured by vegetative forage yield). Thus, high vegetative forage yield in these populations is probably achieved

by different mechanisms, one of which may be rapid tillering and initial regrowth resulting in rapid leaf area development. The high correlation of vegetative forage yield with reproductive forage yield and plant height suggests a second mechanism may involve more uniform growth rates that are sustained throughout the growing season.

A plot of the first two principal components revealed a general pattern to fence and sod populations of most sites (Fig. 2). For Minnesota and Iowa sites, fence and sod populations formed nearly distinct clusters, with the only exceptions being MN-17-fence and IA-29-sod. Sod populations generally had lower values of PRIN1 and/ or PRIN2 than their respective fence population. Fence populations averaged 7.6% higher in reproductive forage yield, 9.5% higher in vegetative forage yield, 6.0% taller, 8.4% wider plant diameter, 4.7% higher regrowth vigor, and 6.9% higher frequent-harvest forage yield (Table 3). For plant height and regrowth vigor, differences between fence and sod populations were consistent across the three states. For all forage yield variables and plant diameter, the difference between fence and sod populations at Iowa sites was one-third to one-half the difference observed for Minnesota and Wisconsin sites. This resulted from a greater among-state variability for sod populations compared with fence populations, which tended to be more uniform among states. Wisconsin sites were the most variable in phenotype, particularly for sod populations, which did not tend to

Table 3. State means for fence or sod populations of smooth bromegrass, averaged across two replicates, 2 yr, 25 clones, and 12 (WI) or 9 (IA and MN) sites.

State	Reproductive forage yield		Vegetative forage yield		Plant height		Plant diameter		Regrowth vigor		Total forage yield (frequent cutting)	
	Fence	Sod	Fence	Sod	Fence	Sod	Fence	Sod	Fence	Sod	Fence	Sod
	——————— Mg ha^{-1} ———————				——————————— cm ———————————						—— Mg ha^{-1} ——	
Wisconsin	3.86	3.61**	2.20	1.97**	104	98**	67	63**	27	26**	2.79	2.61**
Minnesota	3.81	3.34**	2.12	1.92**	104	98**	67	58**	27	26**	2.86	2.62**
Iowa	3.82	3.71	2.20	2.08**	109	102**	69	66**	26	25**	2.66	2.53*
LSD†		2.0		1.4		2		2		1		0.18
Overall mean	3.83	3.56**	2.18	1.99**	106	100**	67	62**	27	26**	2.77	2.59**

** Mean for fence population significantly different from mean for sod population at $P < 0.01$.
† Approximate values for comparing state means at $P < 0.05$.

cluster together. Furthermore, the general pattern that PRIN1 and PRIN2 scores tended to be lower for sod populations was not observed for several of the Wisconsin cemeteries.

The Minnesota sod populations were lowest in reproductive and vegetative forage yield and plant diameter (Table 3). The relatively low means of the Minnesota populations were not due to their greater geographic distance from the collection site to the test site. Linear regressions of the 30 site means on geographic distance between the test site (Arlington, WI) and the collection sites (Fig. 1) were all nonsignificant for both fence and sod populations ($R^2 = 0.01$ to 0.04). All Minnesota collections were made within or near to the Minnesota River Valley, while most Wisconsin and Iowa populations were collected from upland prairie soils. These results suggest the potential for differential adaptation of smooth bromegrass to lowland river-bottom soils vs. upland prairie soils.

Clones collected from fence and sod habitats did not differ in overall phenotype; on the whole, for 1500 clones, neither habitat resulted in unique phenotypes which were not present in the other habitat (Fig. 3). The most extreme individual-clone phenotypes for plant height, plant diameter, and regrowth vigor were found within both habitats. Differences between population means for fence and sod habitats arose from frequency shifts within the distribution of clonal phenotypes.

There are several, not necessarily mutually exclusive, potential factors leading to this observation. First, the founder population for each cemetery likely gave rise to both fence and sod populations at that site. Smooth bromegrass was most likely introduced during or after the drought of the 1930s, either from direct seeding into the cemeteries or by invasion from neighboring agricultural fields. A single introduction event, or repeated events from a local source of smooth bromegrass, would give rise to a certain level of phenotypic similarity between local fence and sod populations. Indeed, it is most likely that there was a single founder population for each cemetery and that sod populations are represented by a highly selected subset of the founder population, which today is most closely approximated by the fence population. Normalized phenotypic distances support this hypothesis. There was a distinct trend toward lower NPD values for fence–sod pairs from the same cemetery (upper right corner of Table 4) compared with the entire population of fence–sod pairwise distances (lower left corner of Table 4). Furthermore, the maximum NPD for fence–sod pairs from the same cemetery was 1.91, while 14% of the 870 fence-sod pairs from different cemeteries exceeded this value, with a maximum value of 4.90. Fence and sod populations from the same cemetery generally shared a greater phenotypic similarity than fence and sod populations from different cemeteries. While phenotype cannot be used to infer genotype per se, these results suggest a possible common ancestry of fence and sod founder populations at most sites.

Second, bidirectional migration between habitats or directional migration from fence to sod, as discussed earlier, would reduce the likelihood of unique pheno-
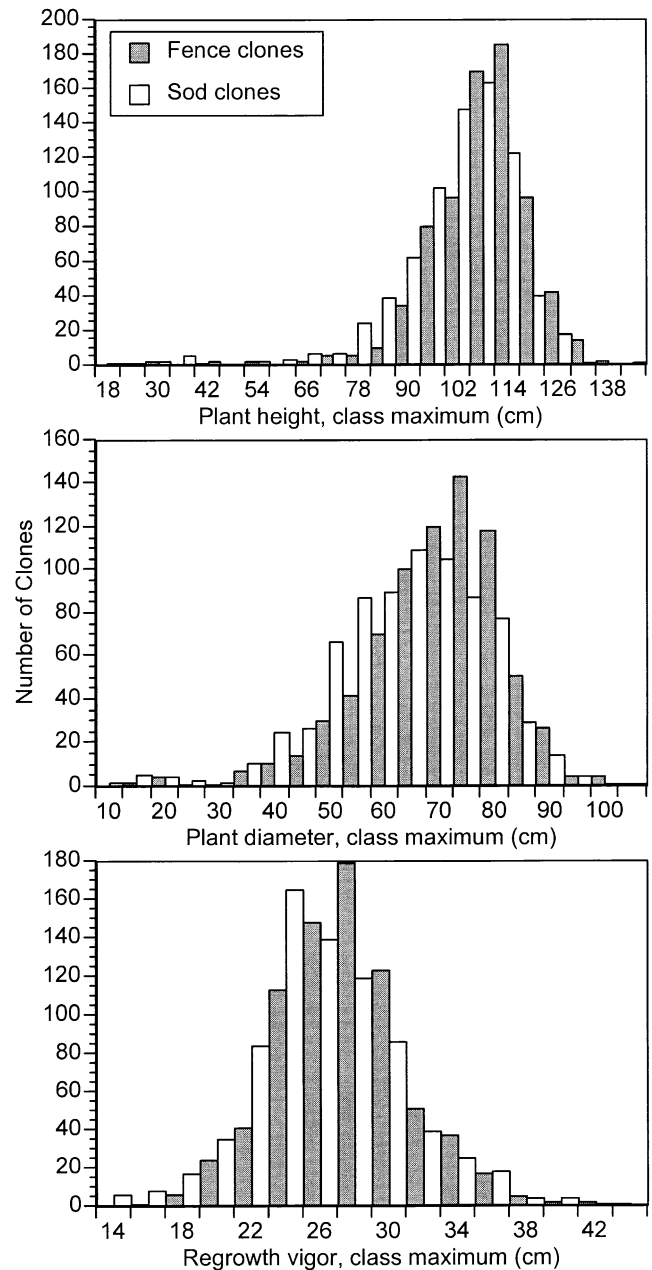

Fig. 3. Frequency distributions of 1500 clone means for fence and sod populations of smooth bromegrass collected from 30 rural cemeteries.

types in each habitat and would create distributions as shown in Fig. 3. New genotypes probably do not arise easily or frequently within either habitat, with seedling recruitment in a dense and vigorous sod as the most likely mechanism for their introduction. Because each cemetery has a well-maintained sod, sexual reproduction is eliminated for all founders and immigrants of sod populations, except in the unlikely event that they migrate into the fenceline. Thus, sexual recombination and transgressive segregation probably have relatively little impact on genetic structure of these populations. It should be recognized that habitat-specific phenotypes may be present in frequencies too low to be detected

Table 4. Means and standard errors of normalized phenotypic distances (NPD) among smooth bromegrass populations collected from fence or sod habitats in three states.†

Habitat	State	Fence			Sod‡		
		WI	MN	IA	WI	MN	IA
Fence	WI	0.81 ± 0.07			0.83 ± 0.20		
Fence	MN	0.70 ± 0.07	0.72 ± 0.15			0.78 ± 0.17	
Fence	IA	0.70 ± 0.04	0.68 ± 0.08	0.56 ± 0.05			0.51 ± 0.07
Sod	WI	1.27 ± 0.07	1.23 ± 0.08	1.29 ± 0.08	1.54 ± 0.14		
Sod	MN	1.17 ± 0.08	1.12 ± 0.08	1.23 ± 0.10	1.17 ± 0.09	0.89 ± 0.10	
Sod	IA	0.84 ± 0.04	0.83 ± 0.05	0.72 ± 0.14	1.09 ± 0.07	0.94 ± 0.08	0.66 ± 0.07

† There were a total of 1770 pairwise NPD values for the 60 populations, divided into state and habitat groups ranging in size from 36 to 144 pairs, represented below the diagonal as state-habitat group means and standard errors.
‡ Values above the diagonal are based only on paired fence and sod populations collected from the same cemetery (n = 12 for Wisconsin, n = 9 for Minnesota and Iowa).

in these collections, or they may arise in the future given sufficient time and proper circumstances.

Third, stabilizing selection would tend to eliminate extreme (and unique) individuals from each habitat because of reduced fitness of extreme individuals at each end of the distribution. However, disruptive selection, acting to accentuate phenotypic differences between the two habitats, precludes stabilizing selection. Furthermore, extremely vigorous individuals (as measured by forage yield, plant height, or plant diameter) are unlikely to have reduced fitness in both habitats.

Fourth, habitat may have little or no effect on natural selection pressures and evolution of phenotype within these populations. The large phenotypic differences between fence and sod populations and their generally consistent level across cemeteries (Table 3; Fig. 2), combined with the strucure of NPD values (Table 4), suggest that this hypothesis is unlikely.

Normalized phenotypic distances among the 60 populations ranged from 0.02 to 5.81. The 30 fence populations tended to be phenotypically similar to each other, with a maximum NPD of 3.84 and mean NPD values for state groups ranging from 0.56 to 0.81 (upper left corner of Table 4). Conversely, the 30 sod populations tended to be more phenotypically distinct from each other compared with the fence populations, with a maximum NPD of 5.81 and mean NPD values for state groups ranging from 0.66 to 1.54 (lower right corner of Table 4). Mean NPD values for specific state groups were always higher for sod populations than for fence populations. The differential in NPD values between fence and sod pairs was greatest for Wisconsin, intermediate for Minnesota, and least for Iowa cemeteries. This reflects the pattern of principal components for which sod populations appeared to be more variable than fence populations in Minnesota and Wisconsin (Fig. 2).

Habitats with greater internal genetic variability would have greater potential to express among-site phenotypic and genetic variability because of a greater potential for the local environment to favor different phenotypes and, ultimately, different genotypes. Both selection and migration could act to maintain greater phenotypic diversity among cemeteries for sod populations compared with fence populations. As discussed above, migration likely is unidirectional from fence to sod habitats, maintaining higher potential levels of genetic variability within sod populations compared with fence populations. In

addition, selection pressure is likely to be greater within sod habitats because of the extreme and potentially stressful nature of the management regime. Smooth bromegrass evolved in natural grasslands without intensive grazing pressure or frequent defoliation. Smooth bromegrass is relatively poorly adapted to frequent mowing, particularly in a competitive environment (Casler, 1988; Eastin et al., 1964; Reynolds and Smith, 1962). Most natural selection is driven by environmental stress per se or by fluctuating environmental stresses (Wright, 1932, 1949), suggesting that the most stressful and/or unstable environment has the greatest potential for selection. Selection may counteract the effects of migration within a particular cemetery sod, resulting in dominance of a relatively few highly fit genotypes. However, these dominant genotypes would likely vary among cemeteries, resulting in genetic variability among cemetery sods.

Eight of the 30 sites (WI-1, WI-3, WI-8, WI-9, WI-10, MN-13, MN-18, and MN-19) had fence and sod populations that were significantly different ($P < 0.05$) for four or five of the six variables measured (Table 5). Five sites (WI-2, WI-5, WI-12, IA-28, and IA-29) had fence and sod populations that were not significantly different for any of the five variables measured. Several Wisconsin sites were exceptions to the generalized phenotypic difference between fence and sod populations described in Table 3. There were two obvious exceptions from Minnesota, sites MN-17 and MN-18.

Sites WI-3 and WI-9 were the most unusual of the Wisconsin sites (Fig. 2; Table 5). In both cases, this was because of the unusual nature of the sod population. Population WI-3-sod had the highest regrowth vigor of all 60 populations, with a mean that was 26.7% higher than its respective fence population and 8.6% higher than the population that ranked second in regrowth vigor (Table 5). All 25 clones of population WI-3-sod showed visible regrowth within 24 h of harvest for all four harvests taken under the infreqent harvest management. No other clone showed visible regrowth until a minimum of 4 d after harvest. Regrowth of WI-3-sod appeared to arise from new tillers, formed during the previous growth cycle, emerged to approximately 4 to 5 cm above the soil surface, and dormant until the release of apical dominance. Despite the rapid initiation of regrowth for plants of WI-3-sod, the long-term growth rate of new tillers was apparently slower than

Table 5. Means of 60 smooth bromegrass populations, defined by state, site, and habitat (fence or sod), averaged over two replicates, 2 yr, and 25 clones.

State	Site	Reproductive forage yield		Vegetative forage yield		Plant height		Plant diameter		Regrowth vigor		Total forage yield (frequent cutting)	
		Fence	Sod	Fence	Sod	Fence	Sod	Fence	Sod	Fence	Sod	Fence	Sod
		— g plant^{-1} —				— cm —						— Mg ha^{-1} —	
WI	1	4.55	3.27**	2.69	2.35	108	100**	73	63**	27	24*	2.55	2.44
WI	2	3.24	3.78	2.42	2.31	101	105	63	65	26	25	2.47	2.19
WI	3	3.84	3.55	2.14	1.65**	113	109	71	64**	27	34**	3.05	2.51**
WI	4	4.17	4.64	2.48	2.57	107	105	70	70	25	22*	3.05	3.05
WI	5	4.20	3.84	2.33	2.32	102	97	66	62	27	28	3.02	2.92
WI	6	3.56	3.20	2.04	1.95	111	93**	67	63	27	27	3.07	2.77
WI	7	3.32	2.92	1.58	1.45	91	93	65	61	26	25	2.58	2.12**
WI	8	3.92	2.59**	2.28	1.75**	106	91**	71	57**	31	27**	2.78	2.67
WI	9	4.34	5.36**	2.25	1.97	97	105**	63	70**	27	19**	3.03	2.79
WI	10	4.45	3.15**	2.11	1.62**	106	93**	70	52**	23	25	2.74	2.73
WI	11	2.92	3.10	1.77	1.37*	97	81**	55	60*	28	27	2.32	2.28
WI	12	3.78	3.93	2.30	2.37	108	108	66	65	27	26	2.84	2.81
MN	13	4.03	3.37**	2.04	1.67	111	101**	69	61**	26	23**	2.58	2.22
MN	14	3.97	3.59	2.42	2.43	106	103	68	60**	28	27	2.88	2.23**
MN	15	3.96	3.62	2.28	1.90	107	99**	69	53**	28	27	3.01	2.72
MN	16	3.65	3.89	2.43	2.46	104	102	69	59**	28	27	2.81	2.47
MN	17	2.65	2.93	1.63	1.77	89	93	48	54*	26	27	2.77	2.68
MN	18	3.61	2.28**	1.98	1.40**	99	89**	67	47**	26	29	3.09	2.79
MN	19	4.43	3.31**	2.46	1.89**	104	98*	73	59**	26	26	3.02	3.13
MN	20	4.25	3.93	2.12	1.87	105	102	71	69	26	23**	2.80	2.95
MN	21	3.69	3.13	1.78	1.85	108	99**	67	58**	27	24	2.81	2.38**
IA	22	3.03	3.51	1.94	1.65	104	99	61	67*	27	27	3.02	2.44**
IA	23	4.36	4.09	2.44	2.42	106	99**	74	63**	29	26**	2.65	2.68
IA	24	4.02	3.89	2.11	2.11	104	95**	67	64	27	22**	2.75	2.93
IA	25	4.07	3.88	2.01	1.47**	114	105**	74	79*	26	24	2.66	2.92
IA	26	4.20	3.54**	2.54	2.44	116	105**	72	60**	24	24	2.85	2.69
IA	27	3.07	3.72**	2.36	2.24	109	106	67	69	26	24	2.73	2.14**
IA	28	3.84	3.51	2.09	2.07	108	107	67	66	27	25	2.14	2.06
IA	29	3.50	3.39	1.93	2.30	108	105	67	65	26	26	2.45	2.46
IA	30	4.26	3.89	2.37	2.00	113	99**	71	62**	26	25	2.72	2.45
LSD(0.05)			0.60		0.42		6		6		3		0.55

* Mean for fence population significantly different from mean for sod population at $P < 0.05$.
** Mean for fence population significantly different from mean for sod population at $P < 0.01$.

that of other populations. Of the 25 highest-ranked clones for regrowth vigor, 13 were from sod populations and 12 from fence populations, and only eight were from WI-3-sod. This population was unremarkable in forage yield and plant diameter, but had the tallest plants at first harvest of the 30 sod populations. The rapid regrowth phenomenon of WI-3-sod was occasionally observed during the frequent-harvest period of 2001–2002, but was much less obvious. The rapid initiation of new tillers and regrowth of WI-3-sod was suggestive of a meadow bromegrass (*Bromus riparius* Rehmann) phenotype. However, random amplified polymorphic DNA (RAPD) markers specific for meadow bromegrass (Ferdinandez et al., 2001) were not found in this population (B.E. Coulman, 2000, personal communication).

Population WI-9-sod had highest reproductive forage yield and the lowest regrowth vigor of all 60 populations ($P < 0.01$ for comparison to all other populations), and was among the highest sod populations in plant height and diameter (Table 5). The low regrowth vigor of this and several other sod populations indicates that rural cemetery sods are not a universal source of smooth bromegrass germplasm with superior regrowth potential. For regrowth vigor, 15 of the 18 lowest-ranked populations vigor were sod populations; for vegetative forage yield, 14 of the 18 lowest-ranked populations vigor were sod populations (Table 5). Within their sod habitat, these smooth bromegrass plants generally ap-

peared to be highly vigorous, with a canopy typically 3 to 8 cm above that of the Kentucky bluegrass sod. However, under the infrequent harvest management, only one sod population exceeded its respective fence population in regrowth vigor (WI-3-sod) and no sod population exceeded its respective fence population in vegetative forage yield. Furthermore, no sod population exceeded its paired fence population in forage yield under the frequent harvest management. Thus, adaptation to frequent mowing does not necessarily confer improved regrowth potential under either a frequent or infrequent harvest management.

Sites MN-17 and MN-18 were unusual for different reasons. Population MN-17-fence ranked 29th or 30th of the 30 fence populations for reproductive and vegetative forage yield, plant height, and plant diameter (Table 5). Conversely, population MN-18-sod ranked 29th or 30th of the 30 sod populations for reproductive and vegetative forage yield, plant height, and plant diameter.

Finally, the low correlations between forage yield for infrequent (1999–2000) vs. frequent (2001–2002) harvests revealed potential adaptive differences between some sod and fence populations. Nine fence populations (WI-1-fence, WI-8-fence, WI-10-fence, MN-18-fence, MN-19-fence, MN-20-fence, IA-23-fence, IA-24-fence, and IA-25-fence) averaged 27.4% higher in reproductive forage yield and 21.9% higher in vegetative forage yield under the infrequent harvest management compared with their respective sod populations. These fence

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populations were similar to their respective sod populations in total forage yield under the frequent harvest management, suggesting that they were unable to express their genetic potential under the frequent harvest management.

CONCLUSIONS

Smooth bromegrass populations from rural cemeteries in Iowa, Minnesota, and Wisconsin are highly variable, with much of the variability arising from phenotypic differentiation within and among sod populations. Results suggest that migration and disruptive selection occur between fence and sod populations at many cemeteries, although it is likely that alleles migrate more frequently from fence to sod than from sod to fence. A few sod populations appear to be highly adapted to their habitat, partly defined by frequent mowing management. Because of the relatively nonstressful nature of the fence habitat and the likely lack of gene migration into the fence habitat, fence populations probably represent relatively minimal changes from the founder populations of smooth bromegrass. Sod populations represent decades of natural selection for tolerance to frequent defoliation without sexual recombination. Thus, with the exception of migrants into the sod habitat, sod and fence plants both represent a subset of the founder population for each cemetery. Plants with tolerance to frequent defoliation were likely present within the founder populations. However, on the basis of the data available, it is not possible to determine if these plants were directly responsible for colonization of the sod habitat or if the entire founder population was present within the sod habitat and the defoliation-tolerant plants were the only plants able to survive long-term frequent defoliation in the sod habitat.

Tolerance to frequent defoliation probably results from rapid initial regrowth following mowing. Frequent mowing may select plants that can rapidly produce new tillers with a small number of leaves, creating just enough leaf area to produce carbohydrate reserves sufficient to support tiller development following the next mowing event. When mowing is withheld from these sod populations for longer periods of time, either on a two- or five-harvests-per-year schedule, a slower long-term growth rate and/or lower photosynthesis rate is likely responsible for their relatively low regrowth vigor and regrowth forage yield. As the regrowth cycle lengthens, sod populations lag further behind fence populations, resulting in similar forage yields or reduced forage yields for the sod populations relative to the fence populations. Nevertheless, some sod populations have similar forage yield potential to fence populations under frequent-harvest management, suggesting a similar short-term growth rate. This germplasm may have potential value for the development of a multipurpose smooth bromegrass cultivar adapted to both infrequent and frequent harvest managements.

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